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(54) PYRROLOBENZODIAZEPINONE DERIVATIVES

(71) We, FUJISAWA PHARMACEUTICAL CO. LTD., of 3, Doschomachi 4-chome, Higashiku, Osaka 541, Japan, a Japanese Company, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

The present invention is concerned with new pyrrolobenzodiazepinone derivatives derived from a new compound produced by a culture of *Streptomyces achromogenes* var. *tomaymyceticus* in a nutrient medium, these new derivatives being active against a number of microorganisms, phages and viruses and are also useful for the treatment of tumors.

The present invention is also concerned with a method of preparing these new pyrrolobenzodiazepinone derivatives.

The new pyrrolobenzodiazepinone derivatives according to the present invention exhibit a high activity against a variety of microorganisms, including gram-positive and gram-negative bacteria, fungi and bacteriophages. The strong virucidal action on some viruses *in vitro* was observed. A further important antibiotic property observed in some of the new derivatives is their ability to inhibit the growth and development of certain transplantable and induced tumors. The antibiotic properties of the new derivatives make them of great utility as therapeutic agents in the treatment of many diseases.

The new compound from which are derived the new derivatives of the present invention, is produced in a fermentation process under controlled conditions in which a hitherto unknown species of *Streptomyces* is used.

The Microorganism

The microorganism useful for the production of this new compound is a newly discovered species of *Streptomyces* isolated from a soil sample collected at Musashi-Koganei in Japan. A culture of the living organism has been deposited with, and added to a permanent stock collection of, the American Type Culture Collection, Rockville, Maryland, in U.S.A. It has now been assigned the number ATCC 21353 and is hereinafter designated as *Streptomyces achromogenes* var. *tomaymyceticus*.

The present invention includes the use of mutants, capable of producing the above-mentioned new compound, produced from the desired organism by various means, such as X-rays, ultraviolet radiation, nitrogen mustards and phage exposure.

For isolating and characterising the microorganism, a portion of the soil sample is shaken in sterile distilled water and plated on Krainsky agar medium. After incubation at 30°C. for 7 days, colonies of *Streptomyces achromogenes* var. *tomaymyceticus* ATCC 21353 are isolated from the medium and then grown on Bennett's agar medium.

Microscopic morphology

The morphology of *Streptomyces achromogenes* var. *tomaymyceticus* ATCC 21353, when grown on Czapek's agar at 30°C. for 10 to 14 days, is given below. The conidium is spherical to oval with a smooth surface. There is a branching and straight or slightly curved long aerial mycelium with thin growth.

Cultural and physiological characteristics

The cultural and physiological characteristics of new strain *S. achrom.* var. *tomaymyceticus* ATCC 21353 in a number of media are listed below. The observation was made after incubation for 10 to 14 days at 30°C. The incubation period and temperature are the same as those described herein unless otherwise indicated.

Czapek's agar—White to pale yellowish colony-like growth; thin growth of powdery white aerial mycelium; no soluble pigment.

Starch-ammonium agar—Faint greyish

growth with powdery, dark grey aerial mycelium; no soluble pigment. There is a weak diastatic action.

Glucose-asparagine agar—White to light ivory colony-like growth; no growth or thin growth of powdery white aerial mycelium, no soluble pigment.

Calcium malate agar—Creamy growth with powdery, white to dark grey aerial mycelium; no soluble pigment. Calcium malate is solubilised.

Tyrosine agar—Thin colourless or light brownish vegetative growth with no aerial mycelium and no soluble pigment.

Bouillon agar—Creamy colony-like growth with no aerial mycelium; brownish soluble pigment produced. Hydrogen sulphide not produced after 7 days' incubation.

Bennett's agar—Light creamy colony-like growth; no aerial mycelium and no soluble pigment. After incubation at 37°C., it produced a brownish colony-like growth with a powdery, dark greyish aerial mycelium and a production of a brown soluble pigment.

Glucose - bouillon—Creamy, colony - like growth with a brown soluble pigment; no growth of aerial mycelium.

Glucose-Czapek's solution—Surface growth poor, colourless, colony-like, with thin growth of powdery white aerial mycelium and no soluble pigment. Nitrate is not or only slightly reduced to nitrite.

Gelatinestab—Creamy growth with no aerial mycelium and no soluble pigment after incubation for 21 days at 15° to 20°C. There is a slight liquefaction of the gelatine.

Litmus milk—The culture grows as a creamy ring at the surface. Faint greyish brown soluble pigment is produced. There is a slight peptonisation but no coagulation.

Potato plug—Greyish creamy vegetative growth with wrinkled surface; thin growth of powdery white aerial mycelium; dark brown soluble pigment produced.

Cellulose agar—There is no growth with ammonium or nitrate ions as nitrogen sources.

Utilisation of Carbon sources

The utilisation of carbon sources was carried out by the Pridham and Gottlieb method after 7 days' incubation at 30°C.

(a) Substrates readily utilised include: Glucose, xylose, mannose, fructose and mannitol.

(b) Substrates moderately well utilised include: Arabinose, rhamnose, sucrose, lactose, trehalose, raffinose and inositol.

(c) Substrate poorly utilised includes: Salicin.

The New Compound

The new compound from which the new derivatives of the present invention are derived is produced when *Streptomyces achromogenes* var. *tomaymyceticus* is grown in a nutrient medium under controlled, submerged, aerobic conditions. A wide variety of nutrient media may be used in the growing stage of the process, an aqueous medium containing an assimilable carbon source and an assimilable nitrogen source, for example a proteinaceous material being employed. Assimilable carbon sources are to be understood as including polyhydric alcohols and mono, di- and poly-saccharides, such as glucose, fructose, sucrose, sugar, brown sugar, starch, corn starch, galactose, dextrin, glycerol and molasses. Proteinaceous materials which can be used include unmodified protein and protein degradation products, particularly products which are formed by the hydrolysis of proteins. Assimilable nitrogen compounds and proteinaceous materials include corn steep liquor, yeast, autolyzed brewer's yeast with milk solids, soya bean meal, peanut meal, cottonseed meal, corn meal, milk solids, pancreatic digest of casein, distillers' solubles, animal peptone liquors, meal extract, peptone, fish meal, yeast extract and meal and bone scraps, as well as inorganic compounds, such as nitrates and ammonium salts. These carbon sources and nitrogen sources need not be used in pure form because the less pure materials, which contain traces of growth factors and considerable quantities of mineral nutrients, are also suitable for use. When desired, these may be mixed with mineral salts, such as sodium chloride and potassium chloride, and with buffering agents, such as calcium carbonate and calcium phosphate. If necessary, a defoaming agent, such as liquid paraffin, fatty oils or silicones, may be added to the fermentating medium.

For maximum growth and development of *Streptomyces achromogenes* var. *tomaymyceticus*, the culture medium, prior to inoculation with the organism, should be adjusted to a pH between about 5.5 and 8.0, preferably between about 6.0 and 7.0. We have observed that, during the growth period of the organism and the production of the new compound, the pH medium should be kept between about 6.0 to 6.5. Maximum production of the new compound appears to occur with the culture medium maintained at a temperature of about 25°C. to 37°C., for a period of 40 to 80 hours. At the end of this period of time, a substantial amount of the new compound is formed.

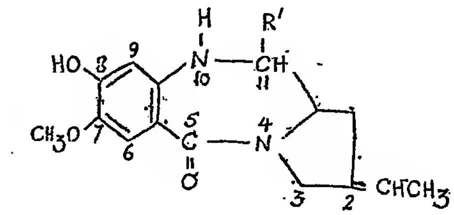
As is preferred for the production of other antibiotics in large amounts, submerged aerobic cultural conditions are those preferred for the production of large amounts of the new compound. For the production of smaller quantities of the new compound, the submerged culture method may be carried out in small flasks or bottles which are either shaken or stirred by suitable mechanical means. However, large volumes of the inoculated nutrient medium can be cultivated in large tanks or

vats customarily employed in the fermentation industry. For the production of large amounts, it is preferable to use the vegetative form of the organism for inoculation of the producton tanks or vats, in order to avoid a growth lag in the production of the new compound. Accordingly, it is desirable first to produce a vegetative inoculum of the organism by inoculating a relatively small quantity of culture medium with the spore form of the organism and then to transfer the vegetative inoculum aseptically to large tanks or vats. The medium in which the vegetative inoculum is produced can be the same as or different from the medium used for the production of the new compound.

Agitation and aeration of the culture mixture may be accomplished in a variety of ways. Agitation may be provided by a propeller or similar mechanical agitation device, by revolving or shaking the fermenter, by various pumping devices or by the passage of sterile air through the medium. Aeration may be effected by injecting sterile air into the fermentation mixture, or it may be provided by spraying, splashing, or pouring the mash into or through the atmosphere.

After the mycelium has been removed from the whole broth by filtration or centrifugation, the new compound can be recovered from the supernatant by extraction or adsorption techniques which are commonly used for the recovery of antibiotics. Extraction may be accomplished by using solvents, preferably water-immiscible organic solvents, including alkyl esters of fatty acids, such as ethyl acetate, and chlorinated hydrocarbons, such as chloroform. Other solvents of similar character can also be used. Combination of these solvents are advantageously employed. Alternatively, the new compound can be recovered from the culture broth with an adsorbing agent, such as diatomaceous earth, activated alumina, silica gel, activated carbon or silicic acid. The new compound is readily eluted from the adsorbent by employing an appropriate polar organic solvent. A suitable method of recovering the new compound from the extract or the eluate comprises the evaporation of the solvent to a relatively small volume and the precipitation of the new compound by the addition of a miscible liquid in which the new compound is insoluble. The new compound is then purified by recrystallisation or chromatography. Solvents which can be used for recrystallisation include aqueous acetone, aqueous methanol and any other solvents in which the new compound is soluble. Adsorbing agents useful for recovering the new compound can also be employed effectively for chromatographic purification. As eluents, there can be used those which can also be employed for recovery of the new compound.

The new compound which is isolated in this manner is obtained in the form of a powder. The precise chemical structure of this new compound has not yet been elucidated but when it is reacted with appropriate alcohols, there are obtained new derivatives according to the present invention, the general formula of which has been found to be:—



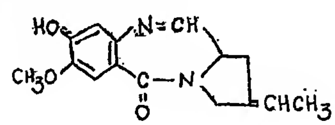
(I)

wherein R' is a C_1—C_6 alkoxy radical.

These derivatives (I) may simply be prepared by dissolving the powdered new compound in and thus reacting it with an appropriate alcohol of the general formula R'H, wherein R' has the same meaning as above, followed by cooling the solution to crystallise out the derivative formed. Examples of alcohols which can be used include aliphatic alcohols containing up to 6 carbon atoms. Preferred examples of such aliphatic alcohols include methanol, ethanol, *n*-propanol and isopropanol. However, other alcohols not specifically mentioned herein can also be used.

The preparation of these derivatives (I) can also be carried out advantageously by reaction with the alcohol in an inert solvent, such as methylene dichloride, chloroform, carbon tetrachloride or ethyl acetate. The reaction temperature is not critical but is preferably between 25°C. and 35°C.

A compound of general formula (I) can be readily converted to a compound of the formula:—

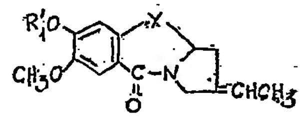


(II)

The elimination of the substituent on the 11-position of the ring may be carried out by dissolving a compound of general formula (I) in a non-alcoholic solvent, such as *n*-hexane, acetonitrile, acetone, chloroform or ethyl acetate, an excess amount of solvent preferably being used. The elimination reaction is preferably performed at ambient temperature but the use of an elevated temperature may also be useful for promoting the reaction and reducing the reaction period. The pricipitate of compound (II) formed in the solution may be separated by conventional

techniques, such as filtration, decantation or centrifugation.

The compounds of general formulae (I) and (II) can be acylated to give compounds of the general formula:—

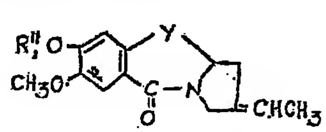


(III)

wherein R'_1 is C_1—C_6 alkyl-carbonyl, aryl C_1—C_6 alkylcarbonyl or aryl carbonyl radical and Y is —NH—CHR— or —N=CH—, R having the same meaning as above.

The acylation reaction may be carried out by mixing the compound with an acylating agent in a solvent, such as pyridine. Any acylating agent which can provide an acyl radical which reacts with a hydroxyl group on the 8-position can be used: these include acids, acid halides, acid anhydrides and acid esters. Examples of such acylating agents include acetic acid, propionic acid, benzoic acid and *p*-bromobenzoic acid and the chlorides, bromides and anhydrides thereof, as well as their methyl and ethyl esters. Such acylating agents are preferably added at ambient temperature or while cooling the solution. Processes comprising mixing a compound of general formula (I) or (II) with an appropriate solvent, cooling the mixture and pouring it into an acylating agent cooled with a mixture of ice and water, result in the formation of a precipitate of the desired acylated compound. These acylated compounds can be crystallised by dissolving the precipitated material in a solvent, such as acetonitrile or methanol and filtering to remove any insoluble material, followed by silica gel chromatography of the filtrate or by washing the filtrate with water. The crystallised compounds may be isolated from solution by conventional techniques, such as filtration.

The compounds of general formulae (I) and (II) can also be alkylated to give compounds of the general formula:—

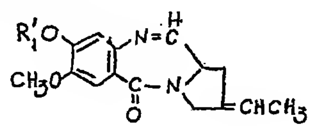


(IV)

wherein R''_1 is a C_1—C_6 alkyl radical and Y has the same meaning as above.

The alkylation reaction may be carried out by mixing the compound with an alkylating agent in a solvent, such as methanol. Any alkylating agent which is capable of providing an alkyl radical which reacts with a hydroxyl group on the 8-position of the compound can be used, such as diazoalkanes and dialkyl sulphates. Examples of such alkylating agents include diazomethane, diazoethane and dimethyl sulphate. This alkylation reaction may be effectively conducted while cooling the reaction mixture. A preferred process for preparing the alkylated compounds comprises placing the reaction solution in a refrigerator, evaporating to dryness, dissolving the residue in methanol, adding ether to the methanolic solution and cooling the ethereal solution to 0°C. The alkylated compounds formed can be isolated by conventional techniques, such as filtration.

The compounds of general formula (III), wherein Y is —NH—CHR—, R having the same meaning as above, can, if desired, be dissolved in a non-alcoholic solvent and thereby converted into a compound of the general formula:—



(V)

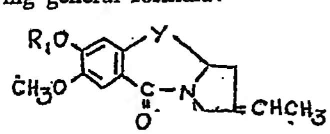
wherein R_1' has the same meaning as above. The conditions used for this conversion reaction are preferably the same as those used for the conversion of compounds (I) into compound (II).

If desired, a compound (II) or (V) or a compound (III) or (IV), in which Y is =N—CH—, can be reacted with an appropriate alcohol, thioalcohol or dialkylamine to give a corresponding compound containing in the 10,11-position, the grouping

—NH—CHR″—

in which R″ is a C_1—C_6-alkoxy, aryl C_1—C_6-alkoxy, C_1—C_6-alkylthio, aryl-C_1—C_6-alkylthio or di-(C_1—C_6-alkyl)-amino radical.

For convenience, the new derivatives of the present invention can be represented by the following general formula:



(VI)

wherein R_1 is a hydrogen atom or a C_1—C_6 alkyl, C_1—C_6 alkyl-carbonyl, aryl-C_1—C_6 alkyl-carbonyl or aryl-carbonyl radical, Y is —N=CH— or —NH—CHR— and R is a C_1—C_6 alkoxy, aryl-C_1—C_6 alkoxy, C_1—C_6 alkylthio, aryl-C_1—C_6 alkylthio or di-(C_1—C_6 alkyl)-amino radical.

The new derivatives (VI) according to the

present invention exhibit a high activity against a number of microorganisms. In the following, there is described the antimicrobial activity of the 11-methoxy compound of general formula (I). The activity of the compound is expressed as its minimum inhibitory concentration (MIC), which is determined by the usual serial agar dilution method. The tests were performed on bacteria using a glucose-bouillon medium and on fungi and yeast using a Sabouraud medium. The test medium was incubated for 24—72 hours at 30°C. and the MIC's are expressed as the concentration of the compound in mcg./ml. which inhibited growth of the organisms.

The following test results were obtained with the 11-methoxy compound of general formula (I):—

Test Organisms	MIC
Staphylococcus aureus 209-P	6.2
Bacillus subtilis ATCC 6633	12.5
Corynebacterium xerosis	25.0
Sarcina lutea	25.0
Escherichia coli	100.0
Pseudomonas aeruginosa	100.0
Proteus vulgaris	100.0
Aspergillus niger	50.0
Penicillium chrysogenum Q-176	25.0
Saccharomyces cerevisiae	50.0
Torula utilis	50.0
Candida albicans	50.0

Results of *in vitro* tests with the 11-methoxy compound against bacteriophages are given below. The tests were performed by adding 1 ml. of a suspension containing 2×10^4 particles of the test phage per ml. in 0.01 M-Tris-HCl buffer (pH 7.2) to each dilution (1 ml.) of samples of the compound to be tested in the above buffer. The mixture (0.1 ml.) was incubated for one hour at 37°C. and poured into a Petri dish containing 1.5% nutrient agar. A phage count was made by the drop-method with the respective host strain, the amount inactivating just 50% of the phages being expressed in mcg./ml.

Test Phages	Concentration inactivating 50% phage activity
Escherichia coli T_1 phage	0.1
Escherichia coli T_2 phage	3.2
Escherichia coli T_3 phage	0.2
Escherichia coli T_4 phage	3.2
Escherichia coli λ phage	1.0
Escherichia coli β phage	12.5
Escherichia coli MS-2 phage	12.5
Bacillus subtilis M-2 phage	0.2
Bacillus subtilis SP-10 phage	0.2
Lactobacillus acidophilus J_1 phage	50.0
Pseudomonas aeruginosa P_2 phage	12.5

Some of the new derivatives have also been found to exhibit an antiviral and antitumor activity. The 11-methoxy compound (I) was found to be active *in vitro* against the DNA virus *Herpes simplex hominis*. In these experiments, 0.1 and 0.05 mg./ml. in a solution of distilled water with 10% dimethyl sulphoxide were mixed in tubes with equal parts of virus suspension in Hanks solution at a dilution of $10^{-3.5}$. After different times of contact at 22°C. the dose of 0.2 ml., which was titered before in Hanks solution as LD_{95}, was injected intraperitoneally into randomised groups of 10 male mice of the NMRJ strain having a weight of 15 to 19 g. As control, 10 mice were injected with 0.2 ml. of the mixed virus suspension, together with a solution of distilled water containing 10% dimethyl sulphoxide as above but without the 11-methoxy compound.

As a result, the 100% mortality of the control group was reduced to 20% after 1 hour and 4 hours and to zero after 6 hours contact. This means that the virulence of this DNA virus is partly or completely destroyed by the action of the 11-methoxy compound.

The 11-methoxy compound also causes a complete inhibition of various transplantable ascites tumors, such as Ehrlich's Carcinoma and Cr. Sarcoma 180, in mice, strain NMRJ, and of the Yoshida Sarcoma strains AH 66 R and AH 130 in Wistar rats. Against the solid Walker Carcino-sarcoma, it is also effective by intratumoral administration. The leukaemia strains L1210-S and L1210-R (6-mercaptopurine-resistant) are partially inhibited: 26—50% (L1210-S) and 51—75% (L1210-R) prolongation of survival time with the well tolerated dose of 0.125 mg./kg. intraperitoneally, 4 applications on 4 consecutive days. In all these tests with transplantable ascites tumor strains, mice or rats were transplanted with a definite amount of cells or cell material in Hanks solution, the amount being one which causes a 100% "take". Randomised groups of 8 animals per dose in rats or 10 animals per dose in mice were first treated, 4 hours after transplantation, with a dose of the 11-methoxy compound, followed by a daily application of the same dose on the 3 following days. Solutions were prepared in triethylene glycol containing 90% distilled water. The single dose given was 0.5 ml. per 20 g. mouse and 1 ml. per 100 g. rat and finally calculated per kg. of mouse and rat.

The activity of the 11-methoxy compound was dose dependent, but a 99% inhibition was still observed with 0.0625 mg./kg. intraperitoneally in the ascites tumors Ehrlich's Carcinoma and Cr. Sarcoma 180 (mouse) and with 0.1 mg./kg. intraperitoneally and 0.05 mg./kg. intraperitoneally in Yoshida Sarcoma AH 66R and Yoshida Sarcoma AH 130 (rat).

All the mentioned doses used in the chemo-

therapeutic experiments were well tolerated by the test animals.

The new derivatives of the present invention can be used as medicaments in the form of pharmaceutical preparations which contain the new derivatives in admixture with a pharmaceutically-acceptable organic or inorganic, solid or liquid carrier suitable for oral or parenteral administration. The solid pharmaceutical preparations may be in the form of capsules, tablets or dragees and the liquid preparations in the form of solutions, suspensions or emulsions. If desired, these preparations can also contain adjuvants, such as preserving agents, stabilising agents, wetting or emulsifying agents and salts for varying the osmotic pressure and buffers. While the dosage of the new derivatives will vary from one derivative to another and also depend upon the age and condition of each individual patient being treated, a daily dose of about 20 mcg./kg. of the compound is generally given for treating diseases against which the antibiotic or its derivatives are useful.

The following Examples are given for the purpose of illustrating the present invention:—

Example 1

The vegetative growth and spores of *Streptomyces achromogenes* var. *tomaymyceticus* ATCC 21353, grown on agar slants, was transferred to a 500 ml. flask containing 100 ml. of the following medium.

Ingredients	Percent by weight
Lactose	3
Meat extract	1
Yeast extract	1
Polypeptone	1
Sodium chloride	0.25

This medium was sterilised and inoculated from agar slants. It was shaken for 3 days at 30°C.

In a 2-ton stainless tank were placed 1000 litres of a fermentation broth having the following composition.

Ingredients	Percent by weight
Lactose	3
Meat extract	1
Yeast extract	1
Polypeptone	1
Sodium chloride	0.25
Potassium dihydrogen phosphate	1.5
Sodium hydrogen phosphate (12 H_2O)	0.43

The pH of the medium was adjusted to 6.1. The culture broth was sterilised by heating it under pressure at about 120°C. for about 30 minutes. The broth was cooled and about 1 ml. of the above inoculant culture was added aseptically. The organism was grown in the broth for 50 to 60 hours at a temperature of 30°C. During the growth period, the broth was stirred and sterile air was blown through the broth at a rate of about 1000 litres of sterile air per minute on a propeller shaker operating at 350 r.p.m.

After the fermentation was completed, the mycelium was removed by centrifugation. The supernatant was treated with about 5 kg. activated carbon, while stirring for 30 minutes. After the mixture had been filtered, the activated carbon was extracted with 100 litres of a mixture of pyridine, ammonia, ethanol and water in a ratio of 10:3:80:10 by warming it at 45°C. for 30 minutes, followed by re-extraction of the activated carbon. The extract was concentrated under reduced pressure at 50°C. and lyophilised to give 1.6 kg. of powder. The powder was washed with about 10 litres of *n*-hexane, dissolved in water and the solution obtained was adjusted to pH 2 to 3. The acidified solution was extracted with four 5-litre portions of chloroform. The chloroform extract was washed with a 5% aqueous solution of sodium bicarbonate, dried over anhydrous sodium sulphate and concentrated under reduced pressure at 50°C. to give an oily residue which was treated with petroleum ether. Filtration of the petroleum ether suspension gave about 20 g. of powder which was dissolved in 100 ml. ethyl acetate and adsorbed on silicic acid in a column and eluted with about 8 litres ethyl acetate. The eluate was concentrated almost to dryness, followed by the addition of about 30 ml. methanol. A precipitate was formed in the methanolic solution by keeping it at −20°C. for 2 days. This was filtered off to give about 1.8 g. of a crude crystalline material which was then dissolved in about 30 ml. warm methanol. The methanolic solution was allow to stand for 2 days at −20°C. to give 1.2 g. pure crystalline 1,2,3,10,11,11a - hexahydro - 2 - ethylidene - 7,11 - dimethoxy - 8-hydroxy - 5H - pyrrolo[2,1-c][1,4]benzodiazepin - 5 - one, melting at 145—146.5°C. (decomposed).

Analysis:

$C_{16}H_{20}N_2O_4$

	C	H	O	N
calculated:	63.16	6.58	21.05	9.21
found:	62.95	6.66	21.25	9.05

The ultraviolet absorption spectrum of this compound in methanol shows maximum peaks at 224 mμ (ϵ=36,000) and 320 mμ (ϵ=3,600), and shoulders at 236 mμ (ϵ=30,000) and 260 mμ (ϵ=9,000), as shown in Fig. 1 of the accompanying drawings.

The infra-red absorption spectrum in a Nujol mull shows bands at 3340, 1640, 1570, 1510, 1425, 1290, 1265, 1210, 1190, 1180, 1070, 830, 800 and 765 cm^{-1}, as can be seen

from Fig. 2 of the accompanying drawings.

Alternatively, 100 litres of the fermentation broth, produced as described above, which had been adjusted with hydrochloric acid to pH 2, were extracted with three 30 litre amounts of chloroform. The chloroform extracts were combined and concentrated to about 10 litres, followed by the addition of 10 litres methanol. The solution was further concentrated to about 300 ml. by the slow removal of methanol. The methanolic solution was placed in a cold refrigerator and the precipitate formed was filtered off and washed with ethyl acetate. The resultant powder was dissolved in warm methanol and allowed to stand in a refrigerator. The precipitated crystalline material obtained was recrystallised from methanol to give about 2 g. 1,2,3,10,11,11a-hexahydro - 2 - ethylidene - 7,11 - dimethoxy-8 - hydroxy - 5H - pyrrolo[2,1-c][1,4]-benzodiazepin - 5 - one.

Example 2

100 Litres of the fermentation broth produced in Example 1 were adjusted with hydrochloric acid to pH 2 and extracted with three 30 litre amounts of ethyl acetate. The extract was evaporated to dryness and the residue obtained was dissolved in a small amount of chloroform. The solution was passed through a column packed with silica gel. The silica gel column was eluted with a mixture of ethyl acetate and chloroform in a ratio of 3:1. The eluate was concentrated to dryness, the residue was mixed with *n*-hexane and the solid material was filtered off, dissolved in chloroform and chromatographed as described above. Evaporation of the eluate gave a powder which was dissolved in ethanol and kept in a refrigerator. The crystalline material formed was recrystallised from ethanol to give pale yellow needles of 1,2,3,10,11,11a - hexahydro - 2 - ethylidene - 7-methoxy - 8 - hydroxy - 11 - ethoxy - 5H-pyrrolo[2.1-c] [1,4]benzodiazepin - 5 - one, melting at 134—136°C. (decomposed).

Analysis:

$C_{17}H_{22}N_2O_4$

	C	H	O	N
calculated:	64.13	6.97	20.10	8.80
found:	63.85	7.02	20.77	8.44

It has ultraviolet absorption peaks, in ethanol, at 225 mμ (ϵ=38,000) and 325 mμ (ϵ=6,700), and shoulders at 235 mμ (ϵ=35,000) and 262 mμ (ϵ=11,000), as shown in Fig. 3 of the accompanying drawings.

It shows infra-red absorption bands in a Nujol mull at 3350, 1640, 1600, 1570, 1513, 1425, 1290, 1265, 1210, 1190, 1160, 1130, 1070, 890, 835, 800, 765 and 710 cm^{-1}, as shown in Fig. 4 of the accompanying drawings.

Example 3

A solution of 1 g. 1,2,3,10,11,11a - hexahydro - ethylidene - 7,11 - dimethoxy - 8-hydroxy - 5H - pyrrolo[2,1-c][1,4]benzodiazepin - 5 - one in an excess of chloroform or ethyl acetate was concentrated to smaller volume and *n*-hexane added to form a precipitate. The precipitate was filtered and washed with ether, while cooling, to give 700 mg. 1,2,3,11a - tetrahydro - 2 - ethylidene - 7-methoxy - 8 - hydroxy - 5H - pyrrolo[2,1-c]-[1,4]benzodiazepin - 5 - one in the form of a pale yellow powder melting at 108—112°C. (decomposed).

Analysis:

$C_{15}H_{16}N_2O_3$

	C	H	O	N
calculated:	66.16	5.92	17.63	10.29
found:	66.04	6.02	17.55	10.41

Example 4

To a solution of 100 mg. 1,2,3,10,11,11a-hexahydro - 2 - ethylidene - 8 - hydroxy - 7,11 - dimethoxy - 5H - pyrrolo[2,1-c][1,4]-benzodiazepin - 5 - one in 5 ml. pyridine was added dropwise 0.2 ml. acetic anhydride, while cooling the solution. The reaction mixture was allowed to stand overnight at ambient temperature and poured into an ice-water mixture. The precipitate formed was filtered off, washed with water, dissolved in 1 ml. methanol and kept in a refrigerator. The resultant precipitate was recrystallised from methanol to give 1,2,3,10,11,11a - hexahydro - 2 - ethylidene - 7,11 - dimethoxy - 8 - acetoxy - 5H-pyrrolo[2,1-c][1,4]benzodiazepin - 5 - one in the form of pale yellow needles melting at 132—133°C.

Analysis:

$C_{18}H_{22}N_2O_5$

	C	H	O	N
calculated:	62.41	6.40	23.10	8.09
found:	62.30	6.53	23.24	7.95

Example 5

To a solution of 300 mg. 1,2,3,10,11,11a-hexahydro - 2 - ethylidene - 7,11 - dimethoxy - 8 - hydroxy - 5H - pyrrolo[2,1-c]-[1,4]benzodiazepin - 5 - one in 5 ml. pyridine was added 400 mg. *p*-bromobenzoic anhydride. The reaction mixture was allowed to stand overnight. The precipitate formed was filtered off, washed with chloroform, washed with 5% aqueous sodium bicarbonate solution and 2N hydrochloric acid and concentrated to smaller volume. This was adsorbed on silica gel in a column and eluted with a mixture of chloroform and ethyl acetate (8:1). The eluate was concentrated to give crude 1,2,3,10,11,11a - hexahydro - 2 -ethylidene - 7,11 - dimethoxy - 8 - *p* - bromobenzoyloxy - 5H - pyrrolo[2,1-c][1,4]benzodiazepin - 5 - one which was dissolved in acetonitrile. Working up the reaction mixture gave white needles of 1,2,3,11a - tetrahydro-2 - ethylidene - 7 - methoxy - 8 - *p* - bromobenzoyloxy- 5H - pyrrolo[2,1-c][1,4]benzodiazepin - 5 - one, melting at 204—205°C.

Analysis:

$C_{22}H_{19}N_2O_4Br$

	C	H	O	N	Br
calculated:	58.02	4.17	14.07	6.15	17.58
found:	58.12	4.25	14.00	6.50	17.58

Example 6

To a solution of 100 mg. 1,2,3,10,11,11a-hexahydro - 2 - ethylidene - 7,11 - dimethoxy-8 - hydroxy - 5H - pyrrolo[2,1-c] [1,4]-benzodiazepin - 5 - one was added dropwise an ethereal solution of diazomethane. The reaction mixture was allowed to stand overnight in a refrigerator and concentrated to dryness. The residue obtained was dissolved in 10 ml. ether, followed by the addition of 10 ml. ethanol and the solution maintained at 0°C. There were obtained pale yellow needles of 1,2,3,10,11,11a - hexahydro - ethylidene - 7,8,11 - trimethoxy - 5H - pyrrolo-[2,1-c] [1,4]benzodiazepin - 5 - one.

Analysis:

$C_{17}H_{22}N_2O_4$

	C	H	O	N
calculated:	64.13	6.97	20.10	8.80
found:	64.33	7.05	20.24	8.60

Example 7

To a solution of 0.54 g. 1,2,3,11a - tetrahydro - 2 - ethylidene - 7 - methoxy - 8-hydroxy - 5H - pyrrolo[2,1-c] [1,4]benzodiazepin - 5 - one in 5 ml. methylene chloride was added 0.26 g. α-toluenethiol. After stirring for 5 hours, the reaction mixture was allowed to stand for 4 days at ambient temperature. After distilling off the methylene chloride under reduced pressure at a temperature of less than 50°C., a yellow powder was obtained which was purified by silica gel thin layer chromatography to give 0.14 g. 1,2,3,10,11,11a - hexahydro - 2 - ethylidene-7 - methoxy - 8 - hydroxy - 11 - benzylthio-5H - pyrrolo[2,1-c] [1,4]benzodiazepin - 5-one. This was recrystallised from benzene to give a pure crystalline material, melting at 143—145°C. (decomposed).

Analysis:

$C_{22}H_{24}N_2O_3S$

	C	H	N
calculated:	66.72	6.11	7.07
found:	66.70	6.00	6.52

Example 8

To a solution of 0.27 g. 1,2,3,11a - tetrahydro - 2 - ethylidene - 7 - methoxy - 8-hydroxy - 5H - pyrrolo[2,1-c] [1,4]benzodiazepin - 5 - one in 5 ml. methylene chloride was added 1.5 ml of ethyl mercaptan. The solution was left to stand for 6 days and then concentrated under reduced pressure to give a residue which was dissolved in water. Methylene chloride was added to form a methylene chloride layer, which was separated, washed with water and dried over anhydrous magnesium sulphate. After distilling off the solvent, there were obtained 0.2 g. yellow crystals of 1,2,3,10,11,11a - hexahydro - 2-ethylidene - 7 - methoxy - 8 - hydroxy - 11-ethylthio - 5H - pyrrolo[2,1-c] [1,4]benzodiazepin - 5 - one, melting at 70—74°C. (decomposed).

Analysis:

$C_{17}H_{22}N_2O_3S$

	N
calculated:	8.65
found:	8.15

Example 9

To a solution of 0.54 g. 1,2,3,11a - tetrahydro - 2 - ethylidene - 7 - methoxy - 8-hydroxy - 5H - pyrrolo[2,1-c] [1,4]benzodiazepin - 5 - one in 5 ml. methylene chloride was added a solution of 1.2 g. dimethylamine. After stirring for 5 hours, the reaction mixture was left to stand for 4 days. The methylene chloride layer was separated, washed with water and dried over anhydrous magnesium sulphate. After a solvent had been removed under reduced pressure, a pale yellowish-brown powder was obtained. This was 1,2,3,10,11,11a - hexahydro - 2 - ethylidene - 7-methoxy - 8 - hydroxy - 11 - dimethylamino-5H - pyrrolo[2,1-c] [1,4]benzodiazepin - 5-one (0.3 g.). It was purified by a silica gel thin layer chromatography to give a pure, yellowish-brown crystalline material, melting at 65—68°C. (decomposed).

Example 10

To a solution of 0.27 g. 1,2,3,11a - tetrahydro - 2 - ethylidene - 7 - methoxy - 8-hydroxy - 5H - pyrrolo[2,1-c] [1,4]benzodiazepin - 5 - one in 5 ml. methylene chloride, there was added 1.5 ml. methanol and the mixture was stirred for 5 days. The reaction mixture was concentrated under reduced pressure to smaller volume and then cooled in a refrigerator. The precipitate formed was filtered off to give a crystalline 1,2,3,10,11,11a - hexahydro - 2 - ethylidene - 7,11 - dimethoxy - 8 - hydroxy - 5H - pyrrolo[2,1-c]-[1,4]benzodiazepin - 5 - one; m.p. 145—146.5°C. (decomposed).

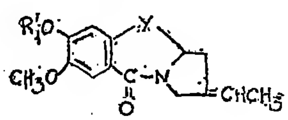
Example 11

An injectable solution in an ampoule was prepared, containing the following ingredients:—

1,2,3,10,11,11a - hexahydro-2 - ethylidene - 7,11 - dimethoxy - 8 - hydroxy-5H - pyrrolo[2,1-c] [1,4]-benzodiazepin - 5 - one	0.02 g.
Ethanol	10.00 ml.
Distilled water	100.00 ml.
1N Sodium hydroxide solution	q.s.
pH value	7.5

WHAT WE CLAIM IS:—

1. Compounds of the general formula:—



wherein R_1 is a hydrogen atom or a C_1—C_6 alkyl, C_1—C_6 alkyl-carbonyl, aryl-C_1—C_6 alkyl-carbonyl aryl-carbonyl radical, Y is a —N=CH— or —NH—CHR— and R is a C_1—C_6 alkoxy, aryl-C_1—C_6 alkoxy, C_1—C_6 alkylthio, aryl-C_1—C_6 alkylthio or di-(C_1—C_6 alkyl)-amino radical.

2. 1,2,3,10,11,11a - Hexahydro - 2 - ethylidene - 7,11 - dimethoxy - 8 - hydroxy - 5H-pyrrolo[2,1-c] [1,4]benzodiazepin - 5 - one.

3. 1,2,3,10,11,11a - Hexahydro - 2 - ethylidene - 7 - methoxy - 8 - hydroxy - 11-ethoxy - 5H - pyrrolo[2,1-c] [1,4]benzodiazepin - 5 - one.

4. 1,2,3,11a - Tetrahydro - 2 - ethylidene-7 - methoxy - 8 - hydroxy - 5H - pyrrolo-[2,1-c] [1,4]benzodiazepin - 5 - one.

5. 1,2,3,10,11,11a - Hexahydro - 2 - ethylidene - 7,11 - dimethoxy - 8 - acetoxy - 5H-pyrrolo[2,1-c] [1,4]benzodiazepin - 5 - one.

6. 1,2,3,11a - Tetrahydro - 2 - ethylidene-7 - methoxy - 8 - *p* - bromobenzoyloxy - 5H-pyrrolo[2,1-c] [1,4]benzodiazepin - 5 - one.

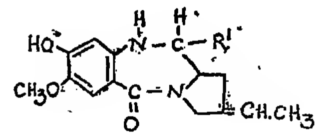
7. 1,2,3,10,11,11a - Hexahydro - 7,8,11-trimethoxy - 5H - pyrrolo[2,1-c] [1,4]benzodiazepin - 5 - one.

8. 1,2,3,10,11,11a - Hexahydro - 2 -ethylidene - 7 - methoxy - 8 - hydroxy - 11-benzylthio - 5H - pyrrolo[2,1-c] [1,4]benzodiazepin - 5 - one.

9. 1,2,3,10,11,11a - Hexahydro - 2 - ethylidene - 7 - methoxy - 8 - hydroxy - 11-ethylthio - 5H - pyrrolo[2,1-c] [1,4]benzodiazepin - 5 - one.

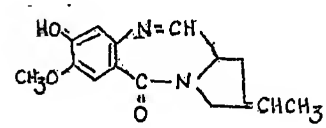
10. 1,2,3,10,11,11a - Hexahydro - 2 - ethylidene - 7 - methoxy - 8 - hydroxy - 11 - dimethylamino - 5H - pyrrolo[2,1-c] [1,4]-benzodiazepin - 5 - one.

11. A process for the preparation of a compound of the general formula given in claim 1, wherein a compound which is produced by culturing *Streptomyces achromogenes* var. *tomaymyceticus* ATCC 21353, or a mutant thereof capable of producing said compound, in a nutrient medium containing assimilable sources of carbon and nitrogen under submerged aerobic conditions until a substantial amount of said compound is accumulated, is, with or without isolation from the culture broth, reacted with an appropriate alcohol to give a compound of the general formula:—



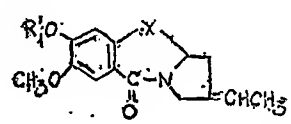
(A)

in which R′ is a C_1—C_6 alkoxy radical; and, if desired, this compound (A) is dissolved in a non-alcoholic organic solvent and thereby converted into a compound of the formula:



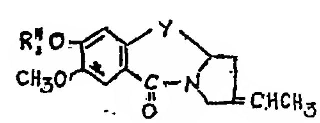
(B)

and, if desired, a compound (A) or (B) is reacted with an acylating agent to give a compound of the general formula:—



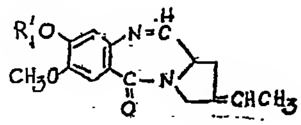
(C)

in which R'_1 is C_1—C_6 alkyl carbonyl, aryl C_1—C_6 alkyl carbonyl or aryl carbonyl radical and Y is —N=CH— or —NH—CHR′—, or, if desired, a compound (A) or (B) is reacted with an alkylating agent to give a compound of the general formula:—



(D)

in which R''_1 is C_1—C_6 alkyl radical and Y is —N=CH— or —NH—CHR′—, wherein R′ has the same meanings as above, and, if desired, a compound (C), wherein Y is —NH—CHR′—, is dissolved in a non-alcoholic solvent and thereby converted into a compound of the general formula:—



(E)

in which R'_1 has the same meaning as above; or, if desired, a compound (B) or (E) or a compound (C) or (D), in which Y is —N=CH—, is reacted with an alcohol, thioalcohol or dialkylamine to give a compound of the general formula given in claim 1, in which R is a C_1—C_6 alkoxy, aryl C_1—C_6 alkoxy, C_1—C_6 alkylthio, aryl C_1—C_6 alkylthio or di-(C_1—C_6 alkyl)-amino radical.

12. Process according to claim 11, wherein the culturing is carried out at a pH of 5.5—8.0.

13. Process according to claim 12, wherein the culturing is carried out at a pH of 6.0—7.0.

14. Process according to any of claims 11—13, wherein the culturing is carried out at a temperature of 25—37°C.

15. A process according to any of claims 11—14, wherein the antibiotic is extracted from the culture medium with a water-immiscible organic solvent.

16. Process according to any of claims 11—14, wherein the antibiotic is removed from the culture medium by adsorption on an adsorbing agent, followed by elution therefrom with a polar organic solvent.

17. Process for the preparation of compounds of the general formula given in claim 1, substantially as hereinbefore described and exemplified.

18. Compounds of the general formula given in claim 1, whenever prepared by the process according to any of claims 11—17.

19. Pharmaceutical compositions comprising at least one compound of the general formula given in claim 1 in admixture with a pharmaceutically-acceptable organic or inorganic, solid or liquid carrier suitable for oral or parenteral administration.

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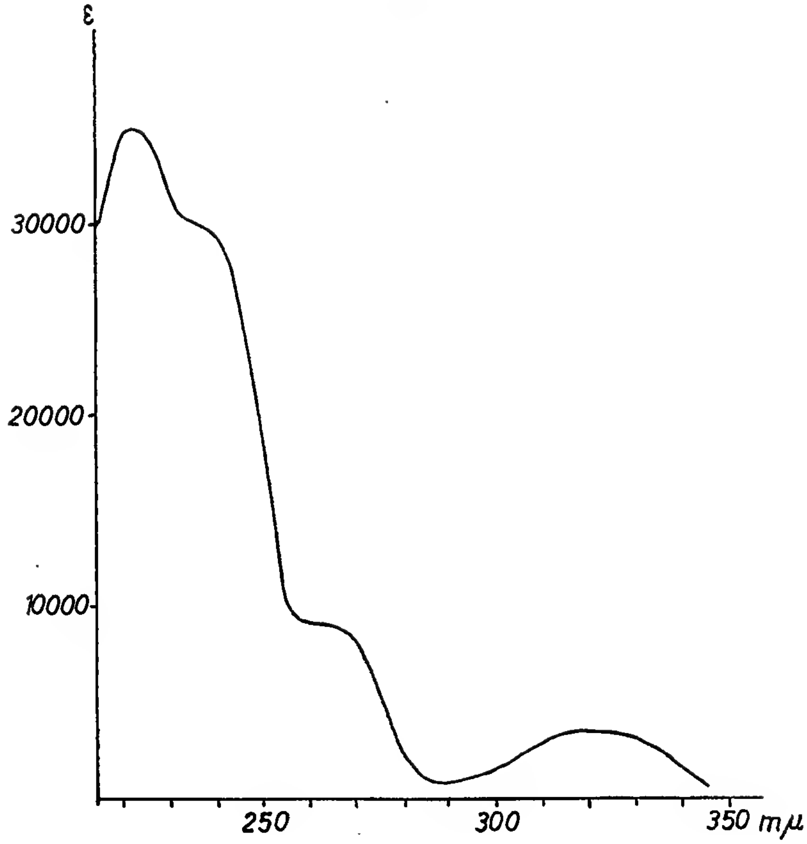
FIG.1



1299198 COMPLETE SPECIFICATION

4 SHEETS *This drawing is a reproduction of the Original on a reduced scale*

Sheet 2

FIG.2

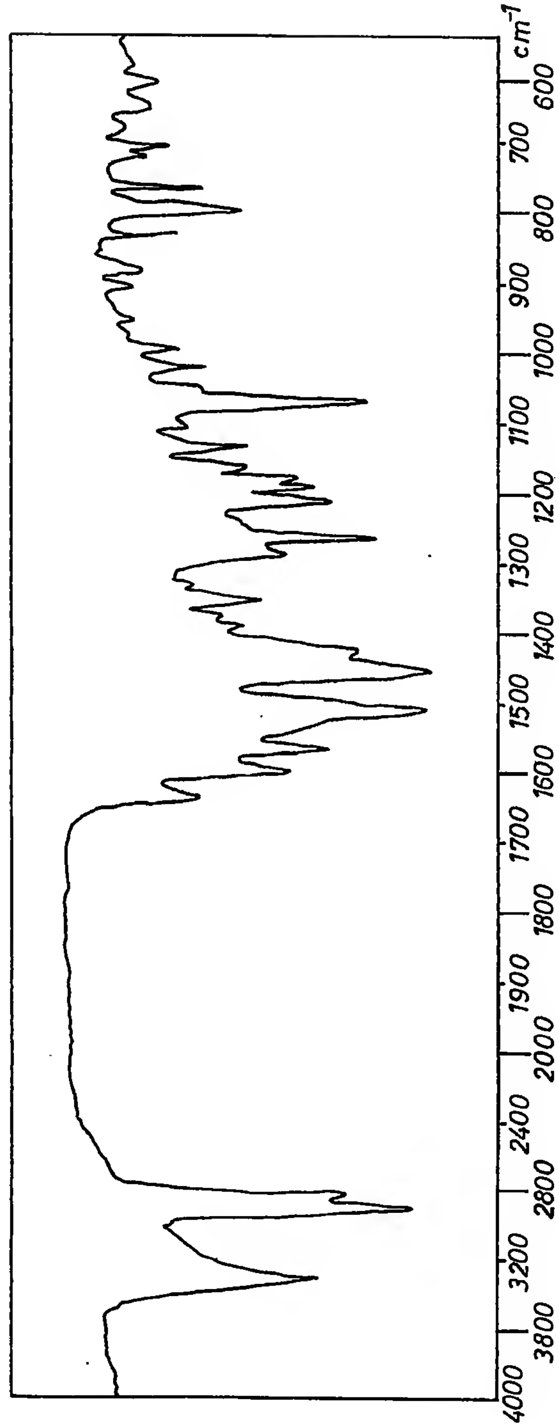
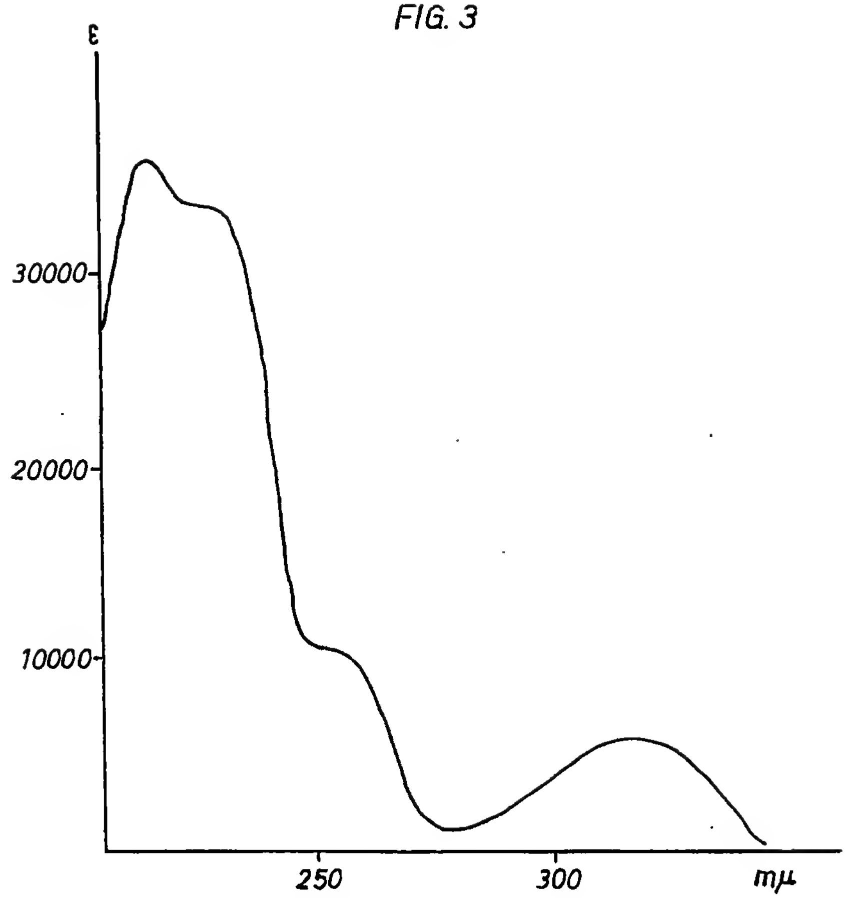


FIG. 3



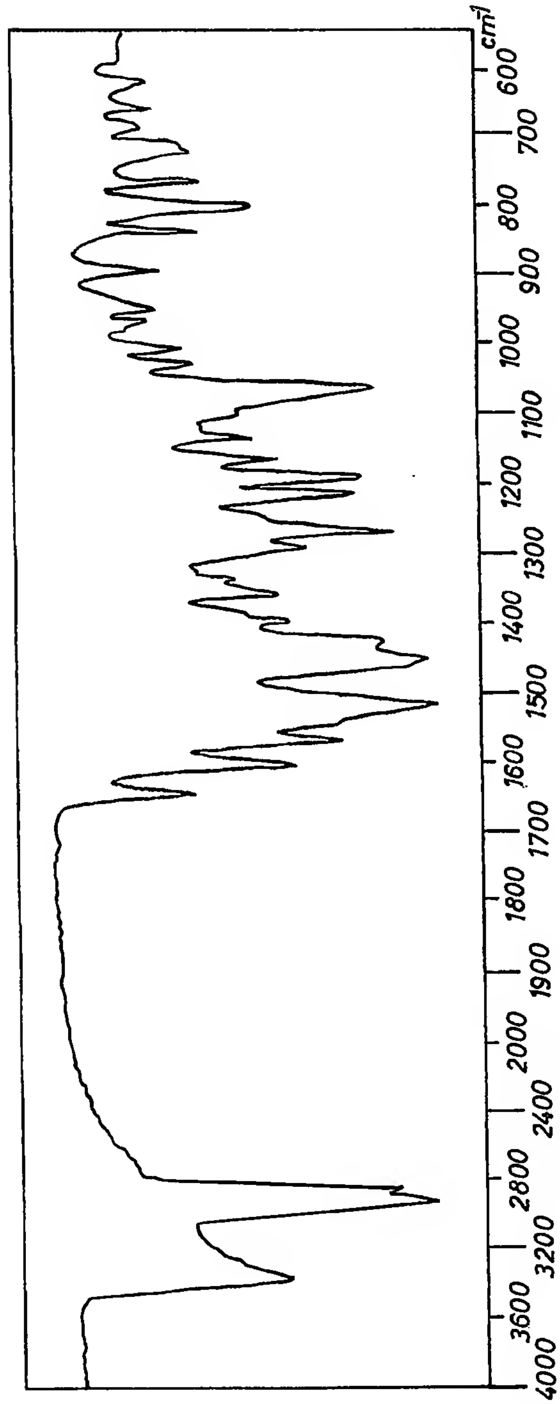

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FIG. 4